



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/SE90/00592 (22) International Filing Date: 14 September 1990 (14.09.90) (30) Priority data: 8903100-9 20 September 1989 (20.09.89) SE (71) Applicant (for all designated States except US): PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only) : KALLAND, Terje [SE/SE]; Odlaresvägen 31, S-240 21 Löddeköpinge (SE). HEDLUNG, Gunnar [SE/SE]; Mårtenstorget 10 C, S-223 51 Lund (SE). DOHLSTEN, Mikael [SE/SE]; Lilla Södergatan 6, S-223 53 Lund (SE). LANDO, Peter [SE/SE]; Sofielundsvägen 44, S-214 34 Malmö (SE).		(74) Agent: THYLÉN, Eva; Leo AB, Box 941, S-251 09 Helsingborg (SE). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>
(54) Title: PHARMACEUTICAL COMPOSITION THAT MAKES CELLS EXPRESSING MHC CLASS II ANTIGENS TARGETS FOR CYTOTOXIC T-CELLS (57) Abstract The present invention concerns a pharmaceutical composition for treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens. The composition comprises a substance making said cells targets for cytotoxic T cells. The substance may be selected from the group consisting of staphylococcal enterotoxins, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.		

PHARMACEUTICAL COMPOSITION THAT MAKES CELLS
EXPRESSING MHC CLASS II ANTIGENS TARGETS
FOR CYTOTOXIC T-CELLS.

The present invention concerns a new agent and a new method for the activation of T cells. Specifically the invention concerns a new agent
5 and method for treating certain cancer forms and autoimmune processes.

Background of the invention

MHC antigens or transplantation antigens, which in man are also called
10 HLA antigens, play an essential part in T cell activation and the concomitant immune response. Thus e.g. protein antigens are dependent on interactions with MHC antigens for presentation to and activation of T cells during a normal immune response. Likewise, MHC dependent presentation of autoantigens are of key importance in T cell dependent au-
15 toimmune processes.

A certain type of MHC antigens, i.e. the so called MHC class II antigens are expressed by a variety of normal cells either constitutionally or after activation (1). Among tumour cells, malignancies of the
20 haematopoietic system commonly express MHC class II molecules.

Although MHC class II antigens are not restricted to tumour cells, they may constitute an attractive target for therapy since most normal cells would be spared.

25 A potent agent capable of eliminating MHC class II antigen expressing cells may therefore be useful both for interruption of autoimmune processes and for the destruction of certain malignant cells.

Summary of invention

30 Accordingly, the present invention concerns a method of treating malignancies involving cells expressing MHC class II antigens, said method comprising administering to a living body a composition including a substance making said cells targets for cytotoxic T cells.

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The invention also comprises a pharmaceutical composition for treating these malignancies. The composition includes a substance making said cells targets for cytotoxic T cells.

- 5 The substance should have the ability of activating the cytotoxic T cells, redirect them to MHC class II antigens irrespective of nominal specificity and thereby inactivate or lyse the MHC class II expressing cells.
- 10 According to the invention it has been found that a preferred substance is of bacterial origin and has the ability to direct T cells to lyse the target MHC class II antigen expressing cell. It is believed that the substance should interact with parts of the T cell receptor complex (TCR) and, more specifically, particular sequences in the T
- 15 cell receptor V beta chains. Preferably the substance or bacterial structure is selected from the group comprising of staphylococcal enterotoxins, SE:s, such as SEA, SEB, SEC, SEC1, SEC2, SEC3, SED or SEE, toxoids active fragments or peptides of these enterotoxins and substances having essentially the same mode of action, such as other
- 20 bacterial products from staphylococcal strains e.g. Toxic shock syndrome toxins (TSST-1) and bacterial exoproteins produced from various other strains, e.g. streptococcal exoproteins and proteins produced by mycoplasma arthritidis, which have similar capacity to interact with certain TCR V-beta sequences. Genetically or chemically modified substances based on the above mentioned structures could also be used.
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An example of a substance which does not fall within the scope of the invention is protein A. This protein emanates from Staphylococcus but it is both structurally and functionally different from the staphylococcal enterotoxins.

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Staphylococcus enterotoxins (SE), which are produced by Grampositive bacteria are a family of molecules of protein structure, that are commonly known for causing food poisoning (2). They are also potent T

35 cell mitogens causing T cell activation and concomitant production of lymphokines (3-5). However, SE unlike most mitogens are strictly dependent on the presence of accessory cells expressing MHC class II molecules (6,7).

Moreover, strong circumstantial evidence has been presented that SEA directly interacts with certain T cell receptor families during their activation of lymphocytes (8,9).

5 It has been previously suggested (WO-A1-89/09619) to use SEA for the treatment of cancer. According to this publication, however, SEA is used for the treatment of blood in vitro (=outside the patients body), and the SEA is removed before the activated cells are reinfused to the patient, whereas the discovery on which the present invention is based
10 discloses that SEA and functionally similar agents can act as bifunctional crosslinkers in vitro. Activating and bringing T cells to tumour cells in the body can be of potential use for the in vivo treatment of certain types of cancer and autoimmune diseases.

15 The agent according to the present invention is preferably in the form of a pharmaceutical composition comprising the substance, together with a pharmaceutically acceptable vehicle or carrier.

The composition is furthermore preferably administered parenterally,
20 and should thus include those adjuvants which are commonly used within this field. Thus the enterotoxin or fragment thereof could be administered in the form of lipid emulsions, or combined with human serum albumin or mixtures of amino acids. The amount of the active component of the composition can vary within broad ranges e.g. 1 ng - 100 mg.

25 In addition to systemic administration it is also possible to use the composition according to the invention for local administration. The composition could e.g. be locally administered in an arthritic joint.

30 Conditions which may be treated by the compositions according to the invention are various forms of autoimmune diseases and tumor diseases e.g. lymphomas and leukemias.

The invention is further illustrated by the following examples which
35 should not be construed as limiting the invention.

Examples

To determine Staphylococcal enterotoxin directed cell mediated cytotoxicity (SDCC) we employed a panel of human anti-HLA-A2 cytotoxic T cell lines as effector cells and the HLA-A2⁻DR⁺ EBV transformed RAJI B-cell line, the R.2.2 HLA-DR⁻ mutant of the RAJI cell line and the HLA-A2⁺DR⁺ EBV transformed BSM B-cell line. The anti-HLA-A2 specific T cell lines were established from primary MLC cultures and repeated restimulations with mitomycin-treated HLA-A2⁺ stimulator cells and recombinant IL-2 (20 units/ml). These T cell lines strongly lysed the specific HLA-A2 expressing target but not irrelevant targets. The effect of Staphylococcal enterotoxin (SE) A, B, C1 and D was studied using SE from Tox Tech (Madison, WI., USA). To demonstrate that HLA-DR is the target molecule in SDCC we used the above described panel of target cells and monoclonal antibodies (mAb) directed to HLA-DR, MHC Class II, W6/32 and CD 23.

Chromium labeling and incubation of the target cells with SEA:

0.75x10⁶ target cells and 150 µCi ⁵¹chromium (Amersham Corp., Arlington Heights, England) were incubated for 45 minutes at 37°C in a volume of 100 µl. The cells were kept in complete medium containing RPMI-1640 medium (Gibco, Paisley, GBR) supplemented with 2.8 % (v/v) 7,5 % NaHCO₃, 1 % sodium pyruvate, 2 % 200 mM L-glutamine, 1 % 1M Hepes, 1 % 10mg/ml gentamicin and 10 % fetal calf serum (FCS, Gibco, Paisley, GBR). After the incubation the cells were washed once in complete medium without FCS and incubated 60 minutes at 37°C and washed and resuspended in complete medium containing 10 % FCS: 5x10³ target cells were added to each well of U-bottom 96-well microtiter plates (Costar, Cambridge, USA).

Cytotoxicity assay

The effector cells were added to the wells at various effector/target cell ratios. The final volume in each well was 200 μ l. Each test was done in triplicate. The plates were incubated 4 hours at 37°C after which the released chromium was harvested using SCS Harvesting frames (Skatron, Norway). The amount ^{51}Cr was determined in a gamma-counter (Cobra Auto-gamma, Packard). The percentage cytotoxicity was computed by the formula

$$\% \text{cytotoxicity} = (X-M)/(T-M) * 100,$$

where X is the chromium release as cpm obtained in the test sample, M is the spontaneous chromium release of target cells incubated with medium, and T is the total chromium release obtained by incubating the target cells with 1% sodium dodecyl sulfate.

Results

Human allospecific T cell lines, which demonstrated exquisite specificity for HLA-A2 showed strong cytotoxicity against the irrelevant HLA-A2⁻DR⁺ RAJI target cell and increased cytotoxicity against the specific HLA-A2⁺DR⁺ BSM target cell, when SEA was added in the assay (Fig. 1 A-B). The T cell mediated SE directed cellular cytotoxicity (SDCC) occurred at very low concentrations of SE, with maximal effect at 10-0.1 ng/ml and half maximal effect was seen at about 0.01 ng/ml (Fig. 1 C). The SDCC phenomenon could be induced by several of the SE. The 5.2 T cell line showed selective reactivity towards SEA, whereas the 5.1 and 11.2 lines reacted with several SE (Fig. 2 A-C). Activation of T cells by the combination of SEA and SEB resulted in an additive increase in cytotoxicity to the RAJI target cell (Fig. 2 C). Blocking studies with monoclonal antibodies (mAb) directed to different cell surface structures demonstrated that the mAb G8, which has recently been shown to interact with a SEA binding site on the HLA-DR molecule, strongly inhibited the SDCC (Table 1. Expt #1). In contrast, the HB 96 mAb, which interacts with a monomorphic MHC Class II determinant, unrelated to the SEA binding epitope and the MHC

Class I and CD23 mAb did not show any inhibitory activity (Table 1. Expt.#1). Preincubation experiments with SE demonstrated that the binding of SE to the RAJI cells and not to the T cell line was a prerequisite for the induction of SDCC against the RAJI cells (Table 1. Expt.#2). The MHC Class II negative RJ2.2.5 mutant of the RAJI cell line was found to be completely resistant to SDCC, while the Class II expressing parental cell line was an excellent target (Table 1. Expt.#3). These observations implicate that HLA-DR is the main target molecule in SDCC.

10

Legend to Figure 1. SEA induced T cell mediated cytotoxicity against RAJI and BSM target cells. The cytotoxicity of an anti-HLA-A2 allospecific T cell line to the RAJI (HLA-A2⁻DR⁺) and BSM (HLA-A2⁺DR⁺) lymphoblastoid cell lines in the presence or absence of 1 ng/ml SEA was examined at various effector/target ratios in a standard 4 hour ⁵¹-chromium release assay and is expressed as % specific cytotoxicity. Figure 1 C demonstrates the dose-response of SEA at concentrations 10⁻⁵ to 10² ng/ml. SEA was added directly into the microtiter wells together with ⁵¹-Chromium labelled RAJI target cells and effector T cells at indicated effector/target cell ratios. Addition of SEA to the target cells in the absence of effector cells did not result in any significant change in the spontaneous release of ⁵¹-chromium.

25

Legend to Figure 2. Induction of SDCC by SEA, SEB, SEC1 and SED. The cytotoxicity of the anti-HLA-A2 allospecific T cell lines 5.2, 5.1 and 11.2 against RAJI target cells in the presence or absence of the various SE at concentration 1 ng/ml was examined in a 4 hour ⁵¹-chromium release assay.

35

Table 1. HLA-DR is the target molecule in SOCC

Effector	Target	Additive		% Cytotox at E/T ratio ^A			
		SE	mAb	A	B	C	
5	Expt.#1.						
	5.2	RAJI	-	-	1	0	0
			+	-	30	19	14
			+	HB96	31	23	16
			+	G8	8	6	3
10			+	W6/32	37	28	19
			+	CD23	40	27	19
Expt.#2.							
	11.2	RAJI	-	-	2	0	0
15			+	-	56	34	14
		RAJI/SE	-	-	33	28	12
	11.2/SE	RAJI	-	-	2	0	0
Expt.#3.							
20	12.1	RAJI	-	-	3	2	3
			+	-	43	30	22
		R12.2.5	-	-	10	11	8
			+	-	12	9	6
		RAJI/SE	-	-	32	24	21
25		R12.2.5/SE-	-	-	12	9	7

^A Cytotoxicity of anti-HLA-A2 allospecific T cell lines against HLA-DR⁺ RAJI cells and MHC Class II negative Raji mutant R.2.2.5 cells. The different mAb were added at the initiation of the culture at an optimal concentration of 20 ug/ml purified ascites Ig (HB 96 and W6/32) or at a ascites dilution 1/300 (G8 and CD23). SE was added to the assay at 0.1 ng/ml (Expt.#1.), 10 ng/ml (expt. #2.) or 1 ng/ml (Expt.#3.). Preincubation of target and effector cells was performed with SE at 1 ng/ml (Expt.#2: RAJI/S and 11.2/SE) or 10 ng/ml (Expt. #3: RAJI/SE and R.2.2.5/SE) for minutes at room temperature. The cells were extensively washed

prior to use in the assay. Addition of SE to the different target cells did not result in any significant change in the spontaneous release of 51 -chromium. The effector/target (E/T) ratios were 20:1, 10:1 and 5:1 in Expt.#1 and #3 and 30:1, 10:1 and 3:1 in Expt.#2.

Composition for injections

1 ng - 100 mg SEA (available from Tox Tech Madison, WI) is dissolved in 0,1 - 5 ml PBS (Phosphate Buffered Saline) and optionally human serum albumin or another conventionally used vehicle.

Injections for administration are intravenous bolus injections including 1-5 ml or continuous infusion of 500 ml during a period of 1-24 h.

For demonstration of in vivo anti-tumor effects of SE the following two experimental approaches are performed:

1. 10^6 murine MHC II⁺ A20 lymphoma cells in 0,3 ml Saline are injected subcutaneously in C57B1/6 mice. The mice are treated with 1 ug of SEA in 0,5 ml Saline intravenously or as control Saline alone. The treatment is given as one dose every week. Tumor growth is followed every day by measurement of total tumor volume. After 4 weeks of therapy the mice are sacrificed.
2. 10^5 murine MHC II⁺ transfected B 16 melanoma cells in 0,3 ml Saline are injected intravenously in C57B1/6 mice. The mice are treated with 1 ug of SEA in 0,5 ml Saline intravenously or as control Saline alone. The treatment is given as one dose every week. Mice are sacrificed after 3 and 4 weeks of therapy and the number of lung metastasis are evaluated.

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Claims

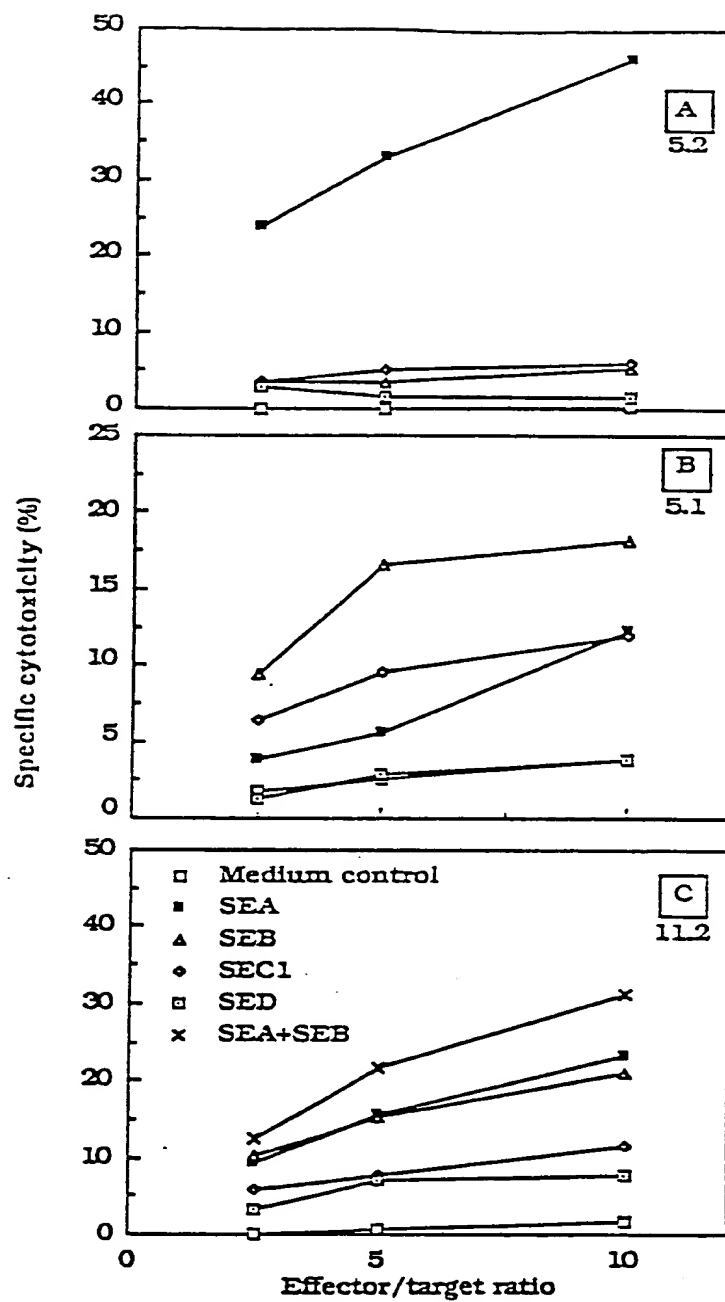
1. Pharmaceutical composition for treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens comprising a substance making said cells targets for cytotoxic T cells.
5
2. Composition according to claim 1 characterized in that said substance has the ability of activating cytotoxic T cells for inactivating or lysing said class II expressing cells.
10
3. Composition according to claim 1 or 2, characterized in that the substance is of bacterial origin and has the capability of interacting with particular sequences in the T-cell receptor V beta chains and directs T-cells to lyse the target MHC class II antigen expressing cell.
15
4. Composition according to claim 3 wherein the substance is selected from the group consisting of staphylococcal enterotoxins, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.
20
25
5. Composition according to claim 4, characterized by being chemical or genetical modifications of said structures.
- 30 6. Composition according to any of the preceding claims, characterized in that the substance is selected from the group Staphylococcal enterotoxin A, B, C, C₁, C₂, C₃, D and E, preferably Staphylococcal enterotoxin A.
- 35 7. Composition according to any of the preceding claims, characterized in that it comprises a combination of two or more enterotoxins, toxoids, active fragments or peptides thereof.

8. Composition according to any of the claims 1-7, characterized in that it is formulated as a parenteral composition.
- 5 9. Composition according to claim 7, characterized in that it comprises parenterally acceptable vehicles, adjuvants and/or carriers.
- 10 10. Method of treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens, comprising administering to a living body a composition including a substance making said cells targets for cytotoxic T cells.
- 15 11. Method according to claim 10, characterized in that the substance is selected from the group consisting of staphylococcal enterotoxins, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.
- 20 12. Method according to claim 11, characterized in that the composition is administered parenterally.
- 25 13. Method according to claim 11, characterized in that the composition is administered systemically or locally.

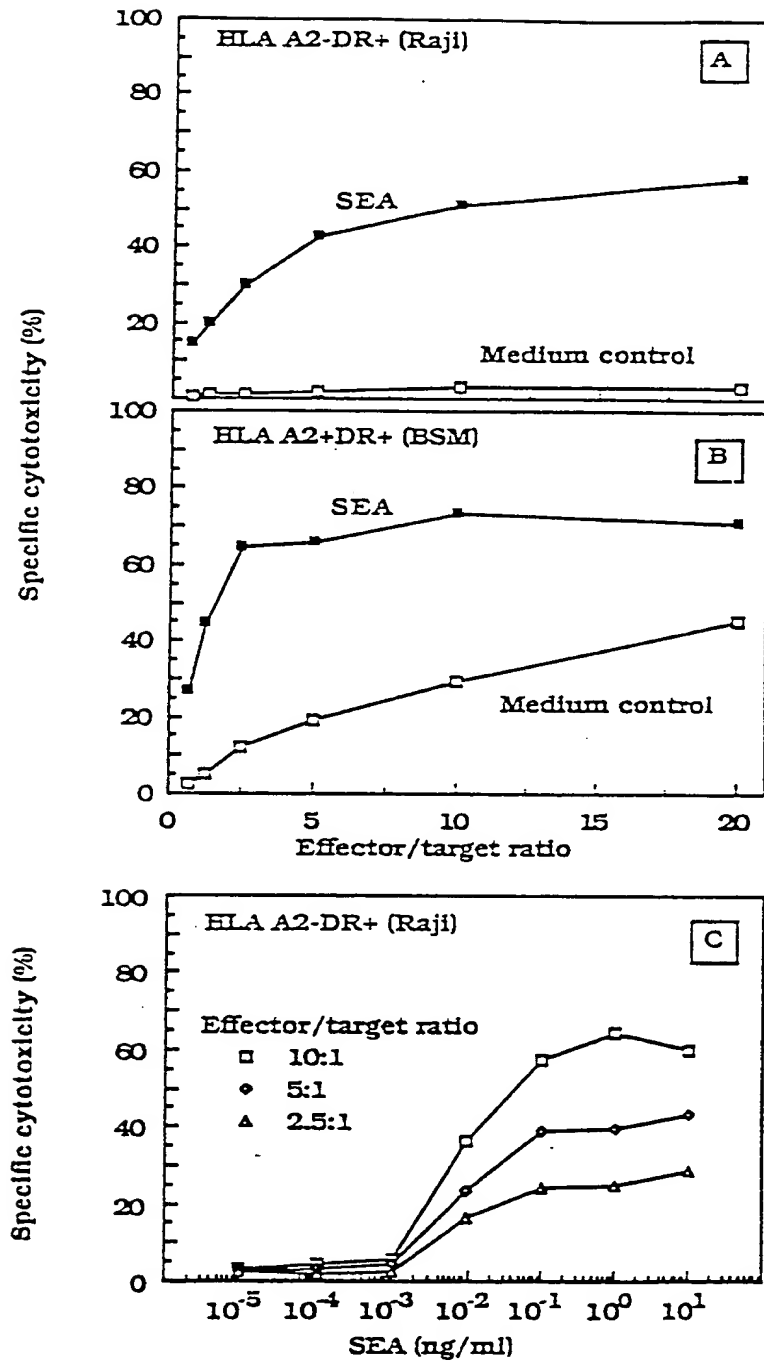
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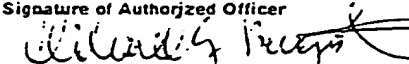
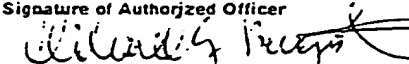
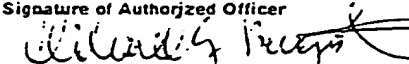


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INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00592

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: A 61 K 39/39, A 61 K35/74														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%; border-bottom: 1px solid black;">Classification System</td> <td style="border-bottom: 1px solid black;">Classification Symbols</td> </tr> <tr> <td style="height: 40px; vertical-align: bottom;">IPC5</td> <td style="height: 40px; vertical-align: bottom;">A 61 K</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in Fields Searched⁸</div> <p>SE,DK,FI,NO classes as above</p>			Classification System	Classification Symbols	IPC5	A 61 K								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category¹⁰</th> <th style="width: 60%;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 30%;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>Nature, Vol. 339, May 1989 J D Fraser: "High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR""", see page 221 - page 223 --</td> <td style="text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>Dialog Information Services, File 154: Medline 83-90, NLM accession no. 89235137, P.M. Stuart et al. "Induction of class II MHC antigen expression in macrophages by Mycoplasma species.", & J Immunol May 1989, 142(10) p3392-3399 --</td> <td style="text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>Dialog Information Service, File 154: Medline 83-90, NLM accession no. 89078460, M. Matthes et al: "Clonal analysis of human T cell activation by the Mycoplasma arthritidis mitogen (MAS)", & Eur J Immunol Nov 1988, 18 (11) p1733-1737 --</td> <td style="text-align: center; vertical-align: top;">1-9</td> </tr> </tbody> </table>			Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	Nature, Vol. 339, May 1989 J D Fraser: "High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR""", see page 221 - page 223 --	1-9	X	Dialog Information Services, File 154: Medline 83-90, NLM accession no. 89235137, P.M. Stuart et al. "Induction of class II MHC antigen expression in macrophages by Mycoplasma species.", & J Immunol May 1989, 142(10) p3392-3399 --	1-9	X	Dialog Information Service, File 154: Medline 83-90, NLM accession no. 89078460, M. Matthes et al: "Clonal analysis of human T cell activation by the Mycoplasma arthritidis mitogen (MAS)", & Eur J Immunol Nov 1988, 18 (11) p1733-1737 --	1-9
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<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="height: 40px; vertical-align: bottom;">14th December 1990</td> <td style="height: 40px; vertical-align: bottom; text-align: center;">1990 -12- 1 9</td> </tr> <tr> <td style="border-bottom: 1px solid black;">International Searching Authority</td> <td style="border-bottom: 1px solid black;">Signature of Authorized Officer</td> </tr> <tr> <td style="height: 40px; vertical-align: bottom; text-align: center;">SWEDISH PATENT OFFICE</td> <td style="height: 40px; vertical-align: bottom; text-align: center;">  Mikael G:son Bergstrand </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	14th December 1990	1990 -12- 1 9	International Searching Authority	Signature of Authorized Officer	SWEDISH PATENT OFFICE	 Mikael G:son Bergstrand				
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International Searching Authority	Signature of Authorized Officer													
SWEDISH PATENT OFFICE	 Mikael G:son Bergstrand													

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A1, 8909619 (ROTHMAN, ULF S E) 19 October 1989, see the whole document --	1-9
A	WO, A1, 8703487 (MCMICHAEL, JOHN) 18 June 1987, see the whole document --	1-9
A	EP, A2, 0223579 (NOVO INDUSTRI A/S) 27 May 1987, see the whole document -- -----	1-9

ANNEX TO THE INTERNATIONAL SEARCH REPORT
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